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Isolation and Chracterization of *Ficus bengalensis* Linn.

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ABSTRACT

Medicinal herbs have a prominent role in human health care. The imposing banyan tree of poetry and legend is a store-house of invaluable remedies for some of the deadliest diseases. *Ficus benghalensis Linn*. synonyms being *Ficus indica Linn*.and *Ficus cotonaeifolia* belongs to the family Moraceae. Present study include phytochemical screening of *Ficus benghalensis* evidenced that chloroform fraction *of Ficus benghalensis Linn* shows comparatively better than aqueous fraction and hydro-alcoholic extract. The isolated phytochemical from chloroform fraction of *Ficus benghalensis Linn* was found as a white amorphous solid compound with melting point of 169-170^{0}C and responded positively to the Salkowski's test and Lieberman-Burchard test for steroids and triterpenes.

Keywords: *Ficus benghalensis,* triterpenes, heamoptysis

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INTRODUCTION

Ficus benghalensis Linn is common in the low country dry regions of Sri Lanka up to an altitude of about 2000 feet. Also occurs in the sub-Himalayan forests and South India and naturalized elsewhere. A very large evergreen tree, 23-34 m tall, with huge spreading limbs supported by aerial roots which later form accessory trunks extending to a large area and stout, softly pubescent branch lets[1,2]. Leaves are Simple, alternate, 10-20 cm long, 5-12.5 cm broad, oval, ovate or orbicular-ovate to oblong, coriaceous, obtusely cuspidate, quite entire, glabrous or pubescent beneath, base rounded, sub chordate or acute, basal veins strong, lateral veins 7-8 pairs, finely reticulate beneath, petioles 1.2-5 cm long, stipules 1.8-2.5 cm long, coriaceous. Flowers are Minute, unisexual, of 3 kinds, males, females and imperfect females (gall flowers) crowded along with bracteoles in the inner walls of fleshy receptacles which are sessile, globes, about 1.8 cm diam., puberulous, arising in axilliary pairs, basal bracts [3], orbicular, spreading. Male flowers: near the mouth of the receptacle, perianth[4], stamen, filament erect. Female are flowers perianth as in the male but shorter, ovary superior, unilocular with a single pendulous ovule, straight or oblique, style excentric, stigma simple. Fruit are Fleshy pericarp and with achenes embedded in them, dark red in colour. Use in Traditional Medicine. The medicinal properties of various parts of the tree have been well known to indigenous physicians. The milky juice is applied externally on pains, bruises, rheumatism, and lumbago and on cracked and inflamed soles of feet[4]. In India, the root is used to treat gonorrhoea, biliousness, dysentery and inflammation of the liver the tips of the aerial roots are also used to relieve persisting vomiting and dysentery. Infusion of the small branches is used for heamoptysis the infusion of the bark is supposed to be a powerful tonic and is considered to have specific properties in the treatment of diabetes.[5, 6]

MATERIAL AND METHODS

Collection and identification

Plant was collected from the India nearby region of Pune during the months of August and September. Taxonomic and ethno medicinal identification of the collected plant done from Director, Botanical survey of India, Pune, Maharashtra.

Preparation of plant material

The aerial parts of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use.1 kg of powdered drugs were packed in soxhlet apparatus and continuously extracted with petroleum ether to defeat the drug. Petroleum ether was removed from the powdered defatted drug, which was then extracted with ethanol (95%). The alcoholic extract thus obtained was further fractioned with hexane, chloroform and ethanol the solvents were removed from each extract and fraction by distillation and the last traces of solvent being removed under reduced pressure[7, 8, 9]. The extracts and fractions were weighed and their % value was recorded which is shown in table 1 and also the physical appearance, colour and odour was evaluated and recorded and thereafter, extracts were stored in refrigerator for further experimental work.

Phytochemical Screening

Qualitative examination of phytoconstituents:

Test for Alkaloids

Dragendorff's Test: To 1 gm of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the orange- red precipitate.

Mayer's Test: To 1 gm of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the cream colored precipitate.

Wagner's Test: To 1 gm of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the reddish brown precipitate.

Test for Carbohydrates:

Molish's Test: To 2gm of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates. Ethanol extract and ethanol fraction give purple colour at the junction of the two liquids.

Fehling's Test: To 1gm of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars. Ethanol extract and ethanol fraction give brick red precipitate.

Test for Glycosides:

Legal Test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red color shows the presence of glycosides. Ethanol extract and its three fractions do not give pink red to red color.

Baljet Test: To 1gm of the test extract, add 1gm of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides. Ethanol extract and its three fractions do not give yellow to orange color.

Test for Tannins and Phenolic Compounds

1. Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give white precipitates.

2. To 1gm of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give dark blue colour.

3. The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red color indicates the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give deep red colour.

Test for Steroids

Libermann-Burchard Test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give bluish-green colour.

Test for Triterpenoids

Noller's Test: Dissolve two or three granules of tin metal in 2ml thionyl chloride solution. Then add 1gm of the extract into test tube and warm, the formation of pink color indicates the presence of triterpenoids.

Ethanol extract, ethanol fraction and chloroform fraction give pink colour.

Test for Flavonoid

Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of Flavonoid.

Ethanol extract, ethanol fraction and hexane fraction yellow colour solution formed, disappears on addition of an acid.

Test for Saponin

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponin.

Spectral Analysis and Structural Elucidation

After preliminary screening by TLC and column chromatography isolated fraction subjected to spectral analysis. For column chromatography silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany) was used. Solvents for chromatography were distilled before use. Thin layer chromatography (TLC) was performed using TLC plates (Silica Gel G-60). Hexane: Ethyl acetate: methanol solvent system with different ratio was found to be the best suitable solvent system for chloroform fraction of Ficus benghalensis Linn and Chloroform, giving good resolution and showing 3 to 4 spots on exposure to iodine vapour by forming the intense brown colour at the spotted area. [10, 11]

UV spectra of the isolated compounds were recorded in methanol over a scanning range of 200-400 nm and λmax of compounds were determined. Spectra were recorded with a Shimadzu 1700 double beam- UV-VIS spectrophotometer. EIMS (electron impact mass spectrum) in positive mode were recorded on Waters Micromass Q-Tof Micro mass spectrometer instrument at SPPU, Pune. The isolate was mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 375- 7500 cm-1 in FT-IR spectroscopy (Model RZX (Perkin Elmer) . ^{1}H and ^{13}C-NMR spectra were recorded on FT-NMR Cryomagnet Spectrometer 400 MHz (Bruker) using TMS as an internal standard at SPPU,Pune India. The solvents used were methanol and DMSO. Chemical shifts ware shown in δ values (ppm) with TMS as an internal reference.

RESULT AND DISCUSSION

Table 1: Characteristics of extract and fraction of *Ficus bengalensis Linn*

Extract/ fraction	Yield (% w/w of powdered drug)	Physical appearance	Colour	Odour
Pet. Ether extract	6.8	Syrupy liquid	Dark green	Pungent aromatic
Ethanol extract	18.8	Syrupy mass	Greenish black	Aromatic
Hexane fraction	6.8	Syrupy mass	Greenish black	Aromatic
Chloroform fraction	5.0	Semisolid mass	Dark green	Characteristically aromatic
Ethanolic fraction	7.2	Semisolid mass	Greenish black	Aromatic

Table 2: Phytochemical presents in extract and fraction of *Ficus bengalensis Linn*

Test for	Ethanolic extract	Hexane fraction	Chloroform fraction	Ethanolic fraction
Alkaloids	+	+	+	+
Carbohydrates	+	-	-	+
Glycosides	-	-	-	-
Phenols and tannins	+	-	-	+
Steroids	+	+	+	+
Terpenoids	+	-	+	+
Saponins	-	-	-	-
Flavonoid	+	+	-	+

(+) = present, (-) = absent

Table 3: Spectral data of isolated compound from chloroform fraction of *Ficus* *bengalensis Linn*

IR (KBr) ν_{max}cm $^{-1}$	^{1}H NMR (in ppm) δ	^{13}CNMR (DMSO)	Ultra Violet Wave Length	EI MS
:3428 (O-H stretching), 2937(C-H stretching), 2852(C-H stretching), 1642 (C=C stretching), 1465 (C-H bend.), 1460 (C-H bend.), 1192 (O-H bend.), 1053 (C-C str.), 739 (CH_2 rocking), 699 (O-H bend.).	δ 5.24 (m, 1H, H-6), δ 4.57 (s, 1H), δ 4.14 (s, 1H), 3.20 (tdd, OH, H-3), δ 1.23 (s, 3H), δ 1.19 (s, 3H), δ 1.06 (s, 3H), δ 0.98 (s, 3H), δ 0.91 (s, 3H).	δ 140.8 (C-22), δ 130.1 (C-5), δ 129.1 (C-23), δ 121(C-6),δ 71.6 (C-3), δ 56.1 (C-4), δ 55.1 (C-5), δ 52.2(C-24), δ 50.10,(C-17), δ 43.8 (C-9), δ 41.2 (C-13), δ 39.4 (C-10), δ 37.7, (C-10), δ 33.4 (C-20), δ 31.7 (C-25), δ 29.1 (C-21), δ 28.1 (C-23), δ 25.1 (C-12), δ 21.8 (C-11, C-25, C-26), δ 15.1 (C-29), δ 12.8(C-27).	257 nm [Petroleum ether (60-80)]	412 [M+, $C_{29}H_{48}O$] 355(101), 311 (49), 301 (49), 279 (71), 219 (60), 200 (65), 175 (95)

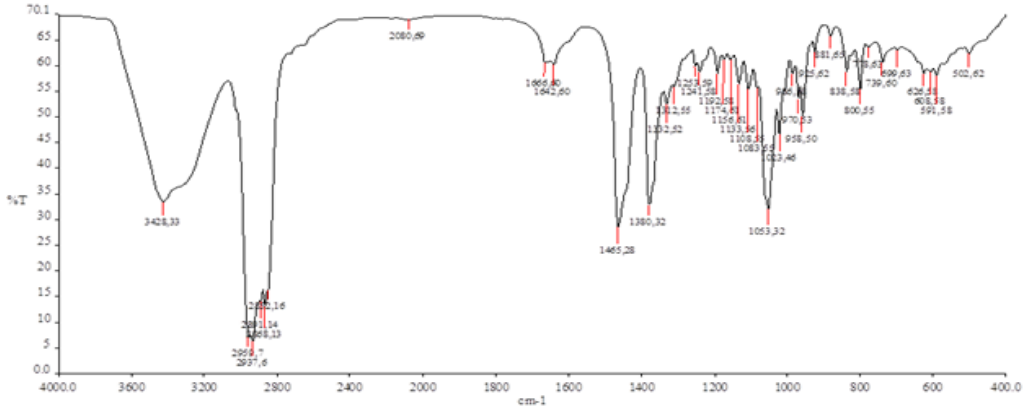

Fig.1: IR spectra of the isolated compound from chloroform fraction of *Ficus bengalensis Linn*

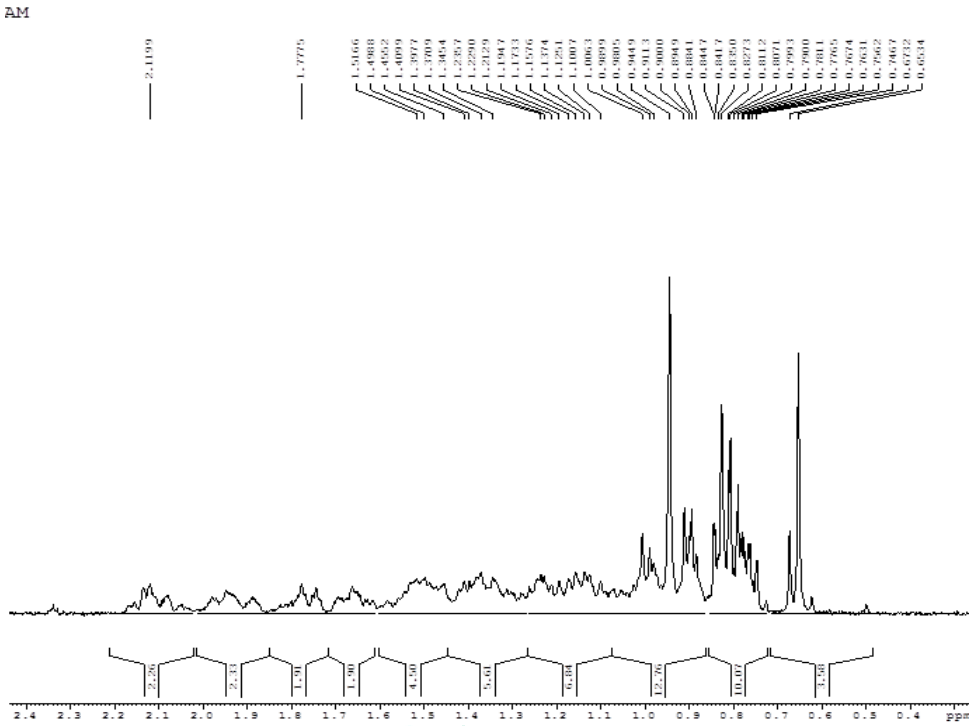

Fig.2: ^{1}H-NMR spectra of the isolated compound from chloroform fraction of *Ficus bengalensis Linn*

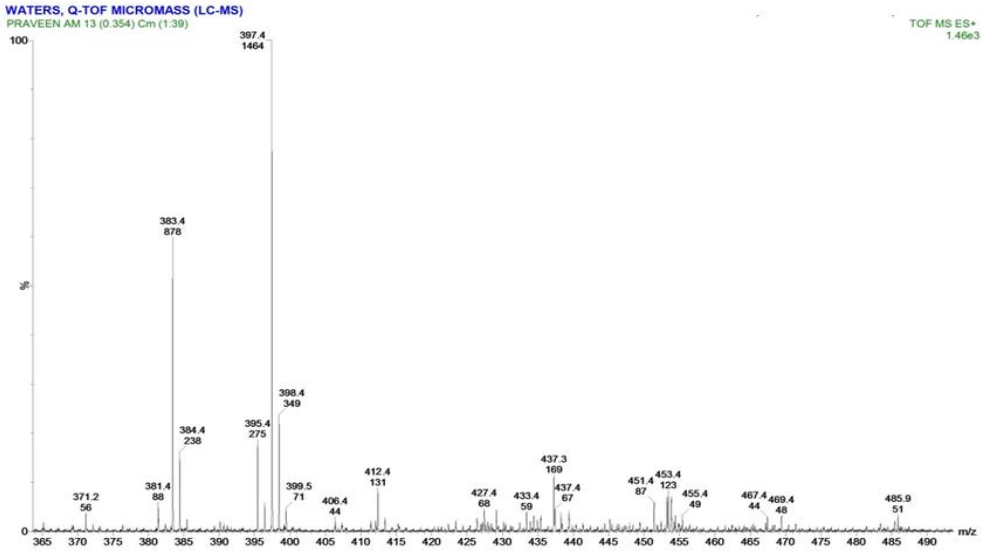

Fig.3: Mass spectra of the isolated compound from chloroform fraction of *Ficus bengalensis Linn*

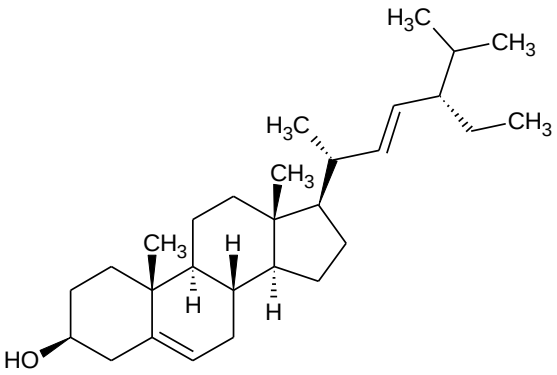

Fig.4: Structure of Stigmasterol isolated from chloroform fraction *of Ficus bengalensis Linn*

Characterization of isolated phytoconstituent from chloroform fraction of *Ficus benghalensis Linn*

Synonym: Stigmasta-5, 22-dien-3β-ol

Molecular formula: $C_{29}H_{48}O$

Molecular weight : 412.69 g/mol

Description: white amorphous solid

Solubility: Soluble in Petroleum ether and Chloroform

R_f value : 0.54 (Chloroform)

M. P. : 169-170^{0}C

CONCLUSION

Chloroform fraction of Ficus benghalensis L shows comparatively better activity than aqueous fraction and hydro-alcoholic extract, hence it forced us to isolate compound present in the chloroform fraction. The isolated phytochemical from chloroform fraction of Ficus bengalensis Linn was found as a white amorphous solid compound with melting point of 169-170^{0}C and responded positively to the Salkowski's test and Lieberman-Burchard test for steroids and triterpenes. The UV λ_{max} value was 257 nm. In IR spectrum of isolated compound, a very intensely broad peak found at 3428 cm^{-1} and moderately intense peak at 1192 and 699 cm^{-1} corresponding to the O-H bond vibrations of hydroxyl group. The out of plane C-H vibrations of the unsaturated part was observed at 881 cm^{-1}. The weakly intense peak was present around 1642 cm^{-1}whichcorrespondsto C=C vibrations. The stretching and bending vibrations of methyl groups were noticed by the intense peak at 2937 cm^{-1} and medium intensity peak at 1465 cm^{-1}. The vibration of the methylene group was evidenced by the peak at 2852 cm^{-1} and the medium peak at 1460 cm^{-1}. The moderately intense peak at 739 cm^{-1} was attributed to the rocking movement of methylene group, corresponding C-C vibration was shown as weak intense peak at 1053 cm^{-1}.

In ^{1}H-NMR spectrum of isolated compound, H-3 proton appeared as a triplet of a double doublet (tdd) at **δ**3.20 (J = 4.5 and 1.1 MHZ) and H-6 olefinic proton showed a multiplet at **S** 5.24. Two olefenic protons appeared downfield at **δ**4.57 (m) and **δ**4.14 (m), which were identical with the chemical shift of H-22 and H-23, respectively of stigmasterol The chemical shift occurred at **δ**1.23, **δ**1.19, **δ**1.06, **δ** 1.00**,** **δ**0.98 and **δ**0.91 of the isolated compound, represent six methyl protons (3H each, s, CH_3).

The ^{13}C-NMR shown recognizable signals at 140.8 and 121ppm, which corresponds to the presence of double bond at C-21 and C-5. The δ value at 71.6 ppm is due to the presence of C-2 β- hydroxyl group. The signal at δ 31.7 and δ 12.8 ppm corresponds to angular carbon atom at C-25 and C-27 respectively. Mass spectrum of isolated compound showed parent molecular ion [M^+] peak at mlz 412 which corresponds to the molecular formula $C_{29}H_{48}O$. This assignment is overall in good agreement for the structure of Stigmasterol derivative containing double bond at 5-position in the nucleus and one five carbon long chain containing methyl groups at different position and one double bond which is also referred with the previously reported similar type of phytocompound as described. The proposed structure of the isolated compound as cited in fig. 4, is derived based on the analysis of different spectra and the name of the compound as per the IUPAC system is (3S,8S,9S,10R,13R,14S,17R)-17[(E,2R,5S)-5ethyl-6methylhept-3-2-yl]-10,13-dimethyl,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol.Various literatures revealed that stigmasterol derivatives have a significant potency to fight against diabetes.

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